# PHYSIOLOGY, ENDOCRINOLOGY, AND REPRODUCTION

## Electrolyte Diets, Stress, and Acid-Base Balance in Broiler Chickens[1]

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**ABSTRACT** We compared the acid-base balance in broiler chickens provided diets containing 2 dietary electrolyte balances (DEB), and administered with either adrenocorticotropic hormone (ACTH) or saline solution. Diets were moderate (174 mEq/kg) or high (241 mEq/kg) DEB formulated by altering Na-K-Cl based on actual analysis. The experiment was designed as a split plot, with the main unit consisting of 4 treatments and the factorial treatment structure arranged in a completely randomized design. Osmotic pumps delivered 8 IU of ACTH in saline/kg of BW per d for 7 d, or the same saline volume as used in ACTH at 1 μL/h for 7 d was implanted on d 35. Venous blood samples were collected on d 35 before the pumps were implanted and on d 42 and 49. Birds fed the high DEB diet exhibited significantly higher $Na^+$ and $Ca^{2+}$ levels than birds provided the moderate DEB diet on d 35. Infusion of ACTH significantly increased ($P \leq 0.05$) hematocrit, hemoglobin, partial pressure of $CO_2$ ($pCO_2$), corticosterone, osmolality, and $HCO_3^-$ and reduced pH, BW, partial pressure of $O_2$ ($pO_2$), and plasma concentrations of $Na^+$ and $Cl^-$ in both diets compared with the control group on d 42. Similarly, the ACTH treatment significantly increased hematocrit, hemoglobin, $Ca^{2+}$, corticosterone, and osmolality and reduced ($P \leq 0.05$) $pO_2$, glucose, and BW on d 49. The diet formulated for high DEB partially lowered $pCO_2$ on d 42. Significant DEB × ACTH interactions were observed for $pCO_2$ and $pO_2$ on d 49. However, there was a reduction in $pO_2$ along with a concomitant increase in erythropoiesis under the ACTH treatment for both diets, compared with the saline control, because of the increased need for $O_2$ to support gluconeogenic energy production. This adaptive response provided greater numbers of erythrocytes and thus a higher amount of circulating hemoglobin to deliver $O_2$ for metabolism. The diet formulated for high DEB partially attenuated the adaptive stress condition in broiler chickens.

**Key words**: electrolyte diet, acid-base balance, stress, adrenocorticotropic hormone, broiler

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## INTRODUCTION

The acid-base balance is influenced by a range of internal and external factors, including the diet, environmental conditions, and metabolism. Collectively, these factors affect moment-to-moment regulation of pH in blood and tissues. Most final metabolites are acids that can be removed from the body by the kidneys and lungs. However, if these metabolites are left unregulated, they can accumulate in the body and alter the acid-base balance from its normal status (Ruiz-Lopez and Austic, 1993).

When an animal experiences conditions that mediate the hypothalamic-hypophyseal-adrenal axis, it will exhibit a series of changes known collectively as physiological stress. It has long been recognized that stress responses are integrally involved with acid-base balance in a number of species (Aguilera-Tejero et al., 2000; Altan et al., 2000; Jochem 2001; Sandercock et al., 2001; Derjant-Li, et al., 2002; Parker et al., 2003; Borges et al., 2003a,b; Yalcin et al., 2004).

The pattern of change in acid-base balance depends on the effects of stressors on the condition and rate of metabolism, respiration, and the mechanism of $H^+$-equivalent ion exchange. However, interpretation of acid-base balance disorders, especially during stress, is complicated by the fact that many relevant variables change simultaneously and in many instances, in opposite directions, depending on the species, sex, age, and type of stressor.

Several management procedures are being performed to minimize the deleterious effects of stress; among these is dietary electrolyte balance (**DEB**; Borges et al., 2003a,b). Addition of salts in the diet or water can beneficially affect the acid-base equilibrium of animals (Hayat et al., 1999; Borges et al., 2004). The effect of acid-base balance on the different metabolic processes of animals is currently an issue discussed by livestock researchers. Recent work has expanded the understanding of the essential role that dietary electrolytes exert on the acid-base balance


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(Derjant-Li et al., 2002). The effect of heat stressors on electrolytes in birds is well known, but more study is required to understand how other stressors induce stress that is likely to influence welfare and performance.

The monovalent minerals sodium ($Na^+$), potassium ($K^+$), and chloride ($Cl^-$) are known as "strong ions" because they exert characteristic effects on the chicken's acid-base homeostasis. These ions play major roles in the synthesis of tissue proteins, maintenance of intracellular and extracellular homeostasis, maintenance of the electric potential of cell membranes, osmotic pressure, and acid-base homeostasis, as well as in enzyme and nerve functioning. A diet that is balanced for electrolytes is highly essential for moment-to-moment physiological and biochemical functions of the body. Borges et al. (2003a) reported the optimal DEB for weight gain to be 236 mEq/kg and for the feed conversion ratio to be 207 mEq/kg, averaging 221.5 mEq/kg. In another study, base excess in heat stress was kept closest to zero by 240 mEq/kg DEB treatments (Borges et al., 2003b).

Recently, we reported the effects of continuous infusion of adrenocorticotropic hormone (**ACTH**) on the acid-base balance in broiler chickens to determine whether acid-base balance is integral to physiological stress (Olanrewaju et al., 2006). Considerable research evidence indicates that DEB can influence the tolerance of poultry to stress (Ahmad and Sarwar, 2006; Lin et al., 2006). The objective of the present study was to determine the effect of ACTH infusion and electrolyte diets on the acid-base balance in broiler chickens. We hypothesized that feeding a diet with the proper DEB would help regulate the acid-base balance in broiler chickens stressed by continuous ACTH infusion.

## MATERIALS AND METHODS

### Bird Husbandry

Four hundred eighty 1-d-old Ross × Ross 708 (Aviagen Inc., Huntsville, AL) male chicks were purchased from a commercial hatchery and randomly distributed into 8 environmentally controlled chambers (60 chicks/chamber). Each chamber was divided into 2 pens each having 30 chicks (2 replicates of 30 chicks in each chamber). Chicks were vaccinated for Marek's disease, Newcastle disease, and infectious bronchitis at the hatchery. Each chamber contained fresh pine shavings, tube feeders, and a nipple watering system having 7 nipples. The birds were provided with tap water and samples were analyzed for mineral composition. Only traces of Na, K, and Cl were found. Birds were provided with a 3-phase feeding program (starter: 1 to 15 d; grower: 16 to 28 d; finisher: 29 to 49 d). Starter feed was provided as crumbles and subsequent feeds were provided as whole pellets. Feed and water were offered ad libitum and lighting was continuous at approximately 10 lx throughout the study. Ambient temperature was maintained at 33°C at the start of experimentation and was reduced as the birds progressed in age to ensure comfort, with a final temperature of 21°C at 35 d and thereafter.

### Treatments

A 2 × 2 factorial arrangement of treatments was used in this study. The 2 factorial components were DEB and ACTH treatment. The DEB was altered by changing the mineral composition of Na-K-Cl by adding sodium chloride, sodium bicarbonate, and potassium chloride to common basal diets, and DEB (in mEq/kg) was calculated based on a previous equation (Leeson and Summers, 2001). The ingredient and nutrient composition of the basal diets is presented in Table 1, and actual values for Na, K, and Cl of the 2 experimental diets are provided in Table 2. The average DEB for the moderate (**M**) and high (**H**) diets were 174 and 241 mEq/kg, respectively. The birds in 4 chambers received the M diet, whereas those in the other 4 chambers received the H diet throughout. Two of the 4 chambers of each diet group were randomly selected to receive either ACTH or saline. The ACTH treatment (**A**) consisted of infusion of 8 IU of ACTH (Sigma-Aldrich Fine Chemicals, St. Louis, MO)/kg of BW per d for 7 consecutive days through implant of osmotic pumps. Control (**S**) birds received pumps that delivered saline at a volume equivalent to that of ACTH-treated birds for 7 consecutive days. The delivery rate of all pumps was 1 μL/h. Sixteen birds (8 birds in each of the 2 pens) per chamber received osmotic pumps and the other birds were extra. The pumps were implanted on d 35 of the study. These 16 birds were marked with spray paint to visually identify whether they possessed an ACTH or saline pump. Puvadolpirod and Thaxton (2000a) have described implantation of the pumps in detail.

### BW, Blood Collection, and Chemical Analyses

Body weights were determined on d 35 (i.e., immediately prior to pump implantation) on d 42 (i.e., after 7 d of continuous ACTH or saline infusion) and on d 49 (i.e., 7 d after the end of the ACTH and saline infusions). Concurrently, 4 birds from each chamber (2 from each replicate pen) were selected at random on d 35 and bled immediately before the pumps were implanted. After blood collection, these birds were removed from the study. These blood samples were analyzed to provide baseline physiological values. On d 42 and 49, 4 birds from each chamber (2 birds from each replication) that possessed pumps were bled and then removed from further experimentation.

Blood samples were collected from the ulnar vein directly into a heparinized (50 IU/mL) monovette syringe. All bleedings were completed within 45 s after the birds were caught. Blood was drawn directly from the syringes into a blood gas/electrolyte analyzer (ABL-77, Radiometer America, Westlake, OH) for immediate analysis of partial pressure of $CO_2$ (**$pCO_2$**), partial pressure of $O_2$

**Table 1.** Ingredient and nutrient composition of basal diets[1]

| Item | Starter (1 to 15 d) | Grower (16 to 28 d) | Finisher (29 to 42 d) |
|---|---|---|---|
| Ingredient (%, as-is) | | | |
| Ground corn | 62.70 | 67.18 | 71.04 |
| Soybean meal (48% CP) | 26.22 | 21.32 | 18.78 |
| Poultry oil | 2.09 | 2.39 | 2.99 |
| Poultry by-product meal | 5.00 | 5.00 | 3.00 |
| Dicalcium phosphate | 1.32 | 1.09 | 1.23 |
| Calcium carbonate | 1.01 | 0.91 | 0.94 |
| DL-Met | 0.24 | 0.22 | 0.18 |
| L-Lys·HCl | 0.02 | — | — |
| L-Thr | 0.04 | 0.04 | 0.03 |
| Mineral and vitamin premix[1] | 0.25 | 0.25 | 0.25 |
| Copper sulfate | 0.05 | 0.05 | 0.05 |
| Zinc sulfate | 0.01 | 0.01 | 0.01 |
| BMD 50[2] | 0.05 | 0.05 | — |
| 3-Nitro[3] | 0.01 | — | — |
| Analyses | | | |
| Calculated ME (kcal/kg) | 3,085 | 3,142 | 3,197 |
| Analyzed CP (%) | 20.3 | 18.4 | 15.5 |
| Calculated TSAA (%) | 0.94 | 0.87 | 0.76 |
| Calculated Lys (%) | 1.25 | 1.11 | 0.97 |
| Calculated Thr (%) | 0.83 | 0.75 | 0.67 |
| Calculated Ca (%) | 0.90 | 0.84 | 0.80 |
| Calculated available P (%) | 1.00 | 0.42 | 0.40 |
| Analyzed Na (%) | 0.03 | 0.03 | 0.02 |
| Analyzed K (%) | 0.78 | 0.69 | 0.63 |
| Analyzed Cl (%) | 0.06 | 0.04 | 0.04 |

[1]Vitamin and mineral premix include (per kilogram of diet): vitamin A (vitamin A acetate), 7,716 IU; cholecalciferol, 2,205 IU; vitamin E (source unspecified), 9.9 mg; menadione, 0.9 mg; $B_{12}$, 0.01 mg; folic acid, 0.6 μg: choline, 379 mg; D-pantothenic acid, 8.8 mg; riboflavin, 5.0 mg; niacin, 33 mg; thiamin, 1.0 mg; D-biotin, 0.06 mg; pyridoxine, 0.9 mg; ethoxyquin, 28 mg; manganese, 55 mg; zinc, 50 mg; iron, 28 mg; copper, 4 mg; iodine, 0.5 mg; selenium, 0.1 mg.

[2]Bacitracin methylene disalicylate (110.2 g/kg, A. L. Laboratories Inc., Ft. Lee, NJ).

[3]3-Nitro-4-hydroxyphenylarsonic acid (50 g/kg, A. L. Laboratories Inc.).

(**$pO_2$**), pH, hematocrit (**Hct**), hemoglobin (**Hb**), and electrolytes ($Na^+$, $K^+$, $Ca^{2+}$, $HCO_3^-$, and $Cl^-$). The pH, $pCO_2$, $pO_2$, and $HCO_3^-$ values were corrected to reflect a body temperature of 41.5°C (Burnett and Noonan, 1974). Anion gap was calculated using the formula $Na^+ - (Cl^- + HCO_3^-)$ (Gupton and Hall, 2001). The mean corpuscular hemoglobin concentration (**MCHC**) was calculated using the standard formula. The needle mounted on each monovette syringe was then removed, a cap was placed over the needle port, and the syringes containing the blood samples were plunged into ice.

After all birds were bled, the iced samples were transferred to the laboratory and centrifuged at 4,000 × *g* for 20 min, and the packed blood cells were then expelled from the syringes. The plunger on each monovette was broken off and the syringe served as a storage vial for

**Table 2.** Ingredient and nutrient composition of experimental diets[1]

| | Starter (1 to 15 d) | | Grower (16 to 28 d) | | Finisher (29 to 42 d) | |
|---|---|---|---|---|---|---|
| Item | High | Moderate | High | Moderate | High | Moderate |
| Ingredient (%, as-is) | | | | | | |
| Basal diet | 99.00 | 99.00 | 98.50 | 98.50 | 98.50 | 98.50 |
| Sodium chloride | 0.22 | 0.39 | — | 0.22 | — | 0.22 |
| Sodium bicarbonate | 0.77 | 0.09 | 0.98 | 0.29 | 1.12 | 0.44 |
| Potassium chloride | — | — | 0.40 | — | 0.20 | — |
| Builder's sand | 0.01 | 0.52 | 0.11 | 0.99 | 0.18 | 0.84 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| Actual analyses | | | | | | |
| Na | 0.30 | 0.19 | 0.25 | 0.19 | 0.25 | 0.19 |
| K | 0.80 | 0.79 | 0.88 | 0.70 | 0.71 | 0.64 |
| Cl | 0.21 | 0.27 | 0.22 | 0.20 | 0.11 | 0.15 |
| DEB[2] | 244 | 167 | 238 | 175 | 242 | 181 |

[1]The high diet was characterized as having a high dietary electrolyte balance (DEB, and the moderate diet was characterized as having a moderate DEB.

[2]Calculated as milliequivalents of Na + K − Cl based on analyzed values (Leeson and Summers, 2001).

the remaining plasma. This procedure ensured that the plasma samples were never exposed to ambient air. Plasma samples were stored at –20°C for later chemical analysis. Plasma samples were removed from the freezer, thawed, and each sample was analyzed for osmolality, corticosterone, (**CS**), cholesterol (**CHOL**), glucose (**GLU**), triglyceride (**TRIG**), and high-density lipoprotein (**HDL**). Concentrations of all plasma chemical constituents, with the exceptions of CS and osmolality, were determined using an autoanalyzer (Ektachem model DT-60, Eastman Kodak Co., Rochester, NY). This analyzer uses the enzymatic procedures described by Elliott (1984). Plasma CS was measured using a universal microplate spectrophotometer (BioTek Instruments Inc., Winooski, VT) with ELISA reagent assay test kits from Assay Designs (EIA-CS kit, Assay Designs Inc., Ann Arbor, MI) according to the manufacturer's instructions. Osmolality was analyzed by a model 3320 microosmometer (Advanced Instruments Inc., Nonwood, MA).

### Statistical Analysis

This study was conducted using a $2 \times 2$ factorial design. The main unit consists of 4 treatments with a factorial treatment structure of 2 ACTH treatments × 2 diets arranged in a completely randomized design, with 2 replicate chambers per treatment. Subunits consisted of measurements taken on 3 d, and the subunit had a repeated-measures structure. Four subsamples were taken for each replication. Separate ANOVA on each date were for a completely randomized design with a $2 \times 2$ factorial structure. Main effects of diet and ACTH infusion and the interaction of these 2 factors were tested using PROC MIXED (SAS, 2004). Comparisons of means at 35, 42, and 49 d were assessed by least significant differences, and statements of significance are based on $P \leq 0.05$. Analyses of variance combined across dates for a split plot were performed to obtain treatment comparisons averaged across dates and to test for treatment interactions with dates. The repeated measures were modeled using a compound symmetry error structure. For some of the responses ($HCO_3^-$, $Cl^-$, CS, HDL, $Ca^{2+}$, $K^+$, MCHC, $Na^+$, CHOL, TRIG, GLU, HDL), the error between dates was not equal. For these responses, a heterogeneous compound symmetry error structure was used for the repeated measures.

## RESULTS

Baseline values were determined on d 35 and treatment effects on d 42 and 49 (i.e., after 7 d of ACTH treatment and 7 d after the end of ACTH treatment, respectively). These values are presented in Table 3. The only difference in baseline values (d 35) was that plasma $Na^+$ and $Ca^{2+}$ concentrations in birds fed the H diet were elevated as compared with birds fed the M diet.

On d 42, pH values in the M + A birds were lower than in the H + S birds but did not differ from the M + S and H + A birds. Values for $HCO_3^-$, $pCO_2$, Hb, Hct, GLU, CHOL, HDL, CS, and osmolality were higher in the M + A and H + A groups than in the M + S and H + S groups. The $pO_2$ of M + S and H + S birds were higher than those of H + A birds, whereas the $pO_2$ of M + A birds did not differ from those of M + S, H + S, and H + A birds. There were neither diet nor ACTH treatment effects on MCHl and $Ca^{2+}$. Levels of $Na^+$ in the M + A and H + A groups were significantly lower than in the M + S birds, whereas $Na^+$ levels in the M + A, H + S, and H + A groups were not significantly different. Levels of $K^+$ in the M + A birds were higher than in the H + S birds, but were not different from levels in the H + A and M + S birds. In addition, $K^+$ levels in the H + S, H + A, and M + S groups did not differ. Chloride ion levels in the M + A and H + A groups were lower than in the M + S and H + S groups. Anion gap levels in the M + A and H + A groups were lower than in the M + S and H + S groups. Levels of TRIG in the M + A birds were higher than in the H + S birds, but were not different from those in the M + S and H + A birds, and TRIG levels in the M + S, H + S, and H + A birds did not differ.

As shown in Table 3, at 7 d after the end of ACTH treatment (d 49), no significant effects of DEB and ACTH treatment were observed for pH, MCHC, CHOL, and TRIG. The values for BW and GLU were lower in the M + A and H + A groups than in the M + S and H + S groups, whereas CS values were higher in the M + A and H + A groups than in the M + S and H + S groups. Levels of $HCO_3^-$ were highest in the H + A birds, lowest in the M + A birds, and did not differ between the M + S and H + S groups. Levels of $pCO_2$ in the H + S and M + A groups were significantly lower than in the H + A birds, but did not differ from the M + S birds. The highest $pO_2$ levels were observed in the H + S birds, but they did not differ from the M + S group. In addition, $pO_2$ levels were lowest in the H + A birds, but did not differ between the M + S and M + A birds. Levels of Hb in the M + A birds were higher than in the M + S and H + S birds, but were not different from the H + A birds. Values for Hct in the M + A birds were significantly higher than in the M + S and H + S groups, but values in the M + S, H + S, and H + A groups were not significantly different. Levels of $Ca^{2+}$ in the H + S birds were lower than in the M + A and H + A birds, but did not differ from the M + S birds. In addition, $Ca^{2+}$ levels in the H + A, M + S, and M + A birds were not significantly different. Levels of $Na^+$ in the M + A birds were higher than in the H + S and M + S birds, but were not different from levels in the H + A birds. Levels of $K^+$ in the M + A birds were significantly lower than in the M + S birds, but levels in the M + A birds were not different from those of the H + S and H + A birds. Values for $Cl^-$ were significantly higher in the M + S and M + A birds than in the H + S and H + A birds. Levels of HDL were higher in the M + S birds than in the M + A and H + A birds, but were not different from those in the H + S birds. In addition, HDL levels were lowest in the M + A birds. Osmolality levels in the M + S and H + S birds were significantly lower than in

**Table 3.** Effects of dietary electrolyte balance (DEB) on broiler chickens stressed by infusion of adrenocorticotropic hormone (ACTH)[1]

| Variable | d 35 | | d 42 | | | | d 49 | | | | Range of SEM |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | H | M + S | M + A | H + S | H + A | M + S | M + A | H + S | H + A | |
| BW (kg) | 2.018 | 2.141 | 2.718$^{a}$ | 2.211$^{b}$ | 2.660$^{a}$ | 2.175$^{b}$ | 3.398$^{a}$ | 2.155$^{b}$ | 3.400$^{a}$ | 2.078$^{b}$ | 0.08–0.16 |
| pH | 7.34 | 7.33 | 7.33$^{ab}$ | 7.29$^{b}$ | 7.36$^{a}$ | 7.33$^{ab}$ | 7.33 | 7.34 | 7.37 | 7.33 | 0.01–0.02 |
| $HCO_3^-$ (mmHg) | 29.7 | 30.8 | 29.5$^{b}$ | 34.7$^{a}$ | 29.5$^{b}$ | 37.0$^{a}$ | 31.5$^{b}$ | 29.2$^{c}$ | 31.1$^{b}$ | 33.1$^{a}$ | 0.98–0.99 |
| Partial pressure of $CO_2$ (mmHg) | 60.3 | 63.9 | 59.8$^{b}$ | 80.8$^{a}$ | 55.8$^{b}$ | 77.4$^{a}$ | 65.1$^{ab}$ | 58.1$^{b}$ | 58.1$^{b}$ | 68.2$^{a}$ | 2.69–4.46 |
| Partial pressure of $O_2$ (mmHg) | 55 | 55.8 | 57.7$^{a}$ | 47.3$^{ab}$ | 54.4$^{a}$ | 39.9$^{b}$ | 49.7$^{ab}$ | 41.2$^{b}$ | 55.8$^{a}$ | 27.0$^{c}$ | 3.24–4.52 |
| Hemoglobin (g/dL) | 7.42 | 7.68 | 7.3$^{b}$ | 8.5$^{a}$ | 7.6$^{b}$ | 8.8$^{a}$ | 7.8$^{b}$ | 8.9$^{a}$ | 7.6$^{b}$ | 8.3$^{ab}$ | 0.25–0.39 |
| Hematocrit (%) | 23.3 | 23.9 | 22.8$^{b}$ | 26.3$^{a}$ | 23.8$^{b}$ | 27.3$^{a}$ | 24.3$^{b}$ | 27.5$^{a}$ | 23.6$^{b}$ | 25.8$^{ab}$ | 0.75–1.19 |
| Mean corpuscular hemoglobin concentration (%) | 32.1 | 32.1 | 32 | 32.1 | 32.1 | 32.2 | 32.1 | 32.3 | 32.2 | 32.2 | 0.06–0.07 |
| $Ca^{2+}$ (mEq/L) | 2.89$^{b}$ | 2.96$^{a}$ | 2.94 | 2.87 | 2.9 | 2.9 | 3.0$^{ab}$ | 3.1$^{a}$ | 2.9$^{b}$ | 3.5$^{a}$ | 0.03–0.07 |
| $Na^{+}$ (mEq/L) | 146$^{b}$ | 148$^{a}$ | 148$^{a}$ | 140$^{bc}$ | 147$^{ab}$ | 138$^{bc}$ | 149$^{b}$ | 158$^{a}$ | 148$^{b}$ | 150$^{ab}$ | 0.71–1.97 |
| $K^{+}$ (mEq/L) | 5.42 | 5.53 | 5.29$^{ab}$ | 5.78$^{a}$ | 4.88$^{b}$ | 5.26$^{ab}$ | 5.43$^{a}$ | 4.63$^{b}$ | 5.12$^{ab}$ | 5.10$^{ab}$ | 0.12–0.17 |
| $Cl^{-}$(mEq/L) | 113 | 114 | 116$^{a}$ | 103$^{b}$ | 114$^{a}$ | 98$^{b}$ | 123$^{a}$ | 126$^{a}$ | 114$^{b}$ | 116$^{b}$ | 1.02–4.46 |
| Anion gap (mEq/L) | 12.03 | 11.49 | 11.7$^{a}$ | 10.92$^{b}$ | 11.7$^{a}$ | 10.51$^{b}$ | 13.08$^{a}$ | 9.6$^{c}$ | 11.31$^{b}$ | 9.31$^{c}$ | 0.61–1.01 |
| Glucose (mg/dL) | 257 | 254 | 255$^{b}$ | 1,047$^{a}$ | 243$^{b}$ | 1,049$^{a}$ | 233$^{a}$ | 182$^{b}$ | 232$^{a}$ | 194$^{b}$ | 0.53–0.65 |
| Cholesterol (mg/dL) | 126 | 129 | 123$^{b}$ | 218$^{a}$ | 114$^{b}$ | 239$^{a}$ | 122 | 110 | 103 | 116 | 4.9–14.2 |
| Triglycerides (mg/dL) | 118 | 106 | 128$^{ab}$ | 238$^{a}$ | 113$^{b}$ | 204$^{ab}$ | 115 | 102 | 107 | 95 | 15–31 |
| High-density lipoprotein (mg/dL) | 99 | 90 | 88.5$^{b}$ | 163.3$^{a}$ | 81.3$^{b}$ | 156.8$^{a}$ | 84.5$^{a}$ | 65.5$^{c}$ | 76.3$^{ab}$ | 72$^{b}$ | 6.79–8.66 |
| Osmolality (mOs/kg) | 314 | 317 | 304$^{b}$ | 334$^{a}$ | 300$^{b}$ | 342$^{a}$ | 302$^{bc}$ | 323$^{a}$ | 306$^{bc}$ | 317$^{ab}$ | 4.37–7.78 |
| Corticosterone (pg/mL) | 700 | 697 | 813$^{b}$ | 26,345$^{a}$ | 830$^{b}$ | 27,126$^{a}$ | 1,002$^{b}$ | 7,646$^{a}$ | 1,487$^{b}$ | 8,893$^{a}$ | 96–345 |

$^{a–c}$Comparative means in a row by day that lack common superscripts are different ($P \leq 0.05$).

[1]Half the birds received the moderate (M) DEB diet and the other half received the high (H) DEB diet throughout the trial. A = 8 IU of ACTH/kg of BW per d for 7 d via osmotic pumps starting on d 35; S = an equivalent volume of saline via pumps at 1 μL/h for 7 d.

**Table 4.** Main effects of dietary electrolyte balance (DEB) and adrenocorticotropic hormone (ACTH) infusion on broiler chickens[1]

| | d 42 | | | | d 49 | | | |
|---|---|---|---|---|---|---|---|---|
| Variable | M DEB | H DEB | Saline | ACTH | M DEB | H DEB | Saline | ACTH |
| BW (kg) | 2.265 | 2.418 | 2.689[a] | 2.193[b] | 2.739 | 2.739 | 3.999[a] | 2.116[b] |
| pH | 7.31 | 7.35 | 7.35[a] | 7.31[b] | 7.34 | 7.35 | 7.35 | 7.34 |
| $HCO_3^-$ (mmHg) | 31.9 | 33.2 | 29.3[b] | 35.9[a] | 30.4 | 32.1 | 31.3 | 31.2 |
| Partial pressure of $CO_2$ (mmHg) | 70.3 | 66.6 | 57.8[b] | 79.1[a] | 61.5 | 63.1 | 61.5 | 63.2 |
| Partial pressure of $O_2$ (mmHg) | 52.5 | 47.2 | 56.1[a] | 43.6[b] | 45.4 | 41.4 | 52.7[a] | 34.1[b] |
| Hemoglobin (g/dL) | 7.87 | 8.19 | 7.45[b] | 8.61[a] | 8.33 | 7.94 | 7.69[b] | 8.59[a] |
| Hematocrit (%) | 24.5 | 25.5 | 23.3[b] | 27.5[a] | 25.9 | 24.7 | 23.9[b] | 26.6[a] |
| Mean corpuscular hemoglobin concentration (%) | 32.1 | 32.1 | 32 | 32.1 | 32.2 | 32.2 | 32.1 | 32.2 |
| $Ca^{2+}$ (mEq/L) | 2.9 | 2.9 | 2.92 | 2.88 | 3.02 | 2.99 | 2.96[b] | 3.05[a] |
| $Na^+$ (mEq/L) | 144 | 142 | 148[a] | 139[b] | 153 | 149 | 148[b] | 154[a] |
| $K^+$ (mEq/L) | 5.53[a] | 5.07[b] | 5.08[b] | 5.52[a] | 5.03 | 5.11 | 5.27[a] | 4.86[b] |
| $Cl^-$ (mEq/L) | 110 | 106 | 115[a] | 101[b] | 124 | 115 | 119 | 121 |
| Anion gap (mEq/L) | 11.28 | 11.1 | 11.67 | 10.71 | 11.33 | 10.15 | 12.04 | 9.44 |
| Glucose (mg/dL) | 657 | 646 | 249[b] | 1,048[a] | 207 | 213 | 233[a] | 188[b] |
| Cholesterol (mg/dL) | 171 | 171 | 58[b] | 79[a] | 116 | 110 | 113 | 113 |
| Triglycerides (mg/dL) | 183 | 159 | 120[b] | 221[a] | 108 | 110 | 111 | 99 |
| High-density lipoprotein (mg/dL) | 126 | 119 | 84[b] | 160[a] | 75 | 74.74 | 80.38[a] | 68.75[b] |
| Osmolality (mOs/kg) | 319 | 321 | 302[b] | 338[a] | 313 | 311 | 304[b] | 320[a] |
| Corticosterone (pg/mL) | 13,579 | 13,978 | 822[b] | 26,735[a] | 4,474 | 5,189 | 1,245[b] | 8,420[a] |

[a,b]Comparative means in a row by day that lack common superscripts are different ($P \leq 0.05$).

[1]Half the birds received the moderate (M) DEB diet and the other half received the high (H) DEB diet throughout the trial. A = 8 IU of ACTH/kg of BW per d for 7 d via osmotic pumps starting on d 35; S = an equivalent volume of saline via pumps at 1 μL/h for 7 d.

the M + A birds but did not differ from those in the H + A birds.

Main effects caused by ACTH treatment and DEB are presented in Table 4. After 7 d of ACTH treatment (i.e., on d 42), main effects of the ACTH treatment and DEB were observed. Levels of $K^+$ in birds on the M DEB diet were significantly higher than those on the H DEB diet. The ACTH treatment caused increases in plasma $HCO_3^-$, $pCO_2$, Hb, Hct, $K^+$, GLU, CHOL, TRIG, HDL, osmolality, and CS levels. Additionally, ACTH decreased blood pH, $pO_2$, $Na^+$, $Cl^-$, and BW. Significant interactions between DEB × ACTH were not observed for any of the variables.

At 7 d (d 49) after the end of ACTH treatment, no effects of DEB were noted for any of the variables. The levels of Hb, Hct, $Na^+$, and $Ca^{2+}$ were elevated in the ACTH-treated groups. Osmolality and CS levels were higher in the ACTH-treated birds than in the saline-infused birds. Additionally, ACTH-treated birds exhibited reduced $pO_2$, $K^+$, GLU, HDL, and BW as compared with saline-treated birds. In addition, interactions of DEB × ACTH were observed for $pCO_2$ and $pO_2$.

## DISCUSSION

Dietary electrolyte balance did not affect BW in the present study, but continuous infusion of ACTH reduced BW under both dietary treatments. This result supports the finding of Lin et al. (2007) that BW of broiler chickens was decreased by either chronic or acute administration of CS. Borges et al. (2003a) reported that during heat stress experiments, the optimal DEB for weight gain was 236 mEq/kg and for FCR was 207 mEq/kg, averaging 222 mEq/kg. However, Veldkamp et al. (2000) reported that DEB (164 vs. 254 mEq/kg), ambient temperature, dietary Arg:Lys ratio, and their interactions did not affect growth performance and carcass yields of male turkeys. Borges et al. (1999) reported growth rate depression in chicks as a result of high values of DEB (354 to 360 mEq/kg). In another study, the base excess in heat stress was kept closest to zero by DEB treatments of 240 mEq/kg (Borges et al., 2003b).

The present results agree with our previous data showing that continuous delivery of ACTH at 8 UI/kg of BW per d for 7 d at a rate of 1 μL/h through a miniosmotic pump stimulated CS release, which induced physiological stress in broiler chickens (Puvadolpirod and Thaxton, 2000a,b,c,d; Olanrewaju et al., 2006; Lin et al., 2007). The ACTH treatment groups exhibited higher levels of $pCO_2$, Hct, Hb, $K^+$, $HCO_3^-$, and plasma osmolality ($\mathbf{p_{osm}}$) along with lower levels of $pO_2$, pH, $Na^+$, $Cl^-$, and BW, compared with the saline control. Increases in Hb and Hct, along with reduced blood oxygen saturation, may be related to the increased metabolic activity needed to meet the energy demands for both maintenance and growth under relatively extreme stressful conditions (Luger et al., 2003). Moreover, increases in Hct can occur as a result of increased muscle activity and the concomitant movement of water from plasma to muscle, leading to an increase in erythropoiesis as a compensatory reaction to the lack of sufficient oxygen in the tissues, possibly because of an impaired oxygen-carrying capacity in the blood.

Stimulation of adrenal steroid secretion by ACTH has been reported to cause an increase in plasma $HCO_3^-$ in the chicken (Kutas et al., 1970). Apparently, hypoxemia coincides with diminished oxygen saturation of the blood (Julian and Mirsalimi, 1992; Wideman et al., 1998), and the concurrent increase in Hct was expected (Shlosberg et al., 1996; Luger et al., 2001). Elevation in Hct may be due to many factors, such as enhanced erythropoiesis because of high levels of CS or diminished plasma volume (Maxwell et al., 1990; Yahav et al., 1997; Luger et al., 2003; Olkowski et al., 2005). Variations in Hct, at least in the

short term, are indicative of changes in blood volume (Takei et al., 1988).

Elevated plasma osmolality in the present study implies that there is greater hydrational stress for the ACTH-treated birds compared with the saline controls. During short-term variations in hydration, Hct is highly positively correlated with changes in plasma osmolality. However, in the longer term, other variables may influence Hct, including age (Gilbert, 1969) and sex (Sturkie and Newman, 1951). High $p_{osm}$ has been reported to be caused by high sodium (hypernatremia), which is the primary contributor; hyperglycemia; uremia; the presence of an unknown molecule; or a combination of these factors (Bhagat et al., 1984; Gennari, 1984). In the present study, this would be hyperglycemia or uremia, because these metabolites are associated with stress in chickens (Siegel, 1995; Puvadolpirod and Thaxton, 2000b,d).

One of the major effects of high blood levels of CS observed in this study was an increase in plasma GLU levels by catabolism of muscle protein, which is driven directly by CS-activated gluconeogenesis. Additionally, ACTH-treated chickens have been reported to exhibit polydipsia and polyuria throughout the stress and recovery periods (Puvadolpirod and Thaxton, 2000d). Plasma osmolality and CS concentration responded to the stressful conditions caused by continuous infusion of ACTH. Dehydration resulted in increased $p_{osm}$ and plasma CS concentration along with other blood metabolic variables (GLU, CHOL, TRIG, and HDL). The results of this study agreed with the possibility of dehydration, and this conclusion is based on our finding of increases in $p_{osm}$ concomitant with an increase in CS concentration. The increase in these 2 factors in broilers exposed to continuous infusion of ACTH suggests problems in balancing body water because of stress induced by continuous infusion of ACTH. Continuous infusion of ACTH may also increase cutaneous water loss and thus contribute to body water imbalance. Thus, it is suggested that, at high ACTH, both a high degree of water loss from the surface together with insufficient drinking leads to some degree of dehydration associated with an increase in $p_{osm}$ and CS concentration. Small increases in $p_{osm}$ will stimulate antidiuretic hormone secretion (Stallone and Braun, 1986; Gray and Erasmus, 1989) and drinking (Kaufman and Peters, 1980). Dehydration-induced changes in $p_{osm}$ are quickly restored when birds regain access to water (Takei et al., 1988).

It has been suggested that programs of selection for high growth rate and muscle yield in broiler chickens may have adverse effects on structural, metabolic, and functional parameters in skeletal muscle, precipitating spontaneous myopathy (Soike and Bergmann, 1998). These perturbations include reductions in the systemic arterial $pO_2$, saturation of Hb with $O_2$ ($HbO_2$), and increased $pCO_2$, leading to respiratory acidosis caused by hydrogen ion ($H^+$, acid) accumulation (Julian and Mirsalimi, 1992; Wideman and Tackett, 2000; Wideman et al., 2000, 2002). A significant increase in Hb concentration was detected in this study. This elevation in Hb is a well-known physiological response to stress, which serves to increase the oxygen-carrying capacity of blood.

The decrease in $Na^+$ concentration might be related to concurrent loss of $Na^+$ and water due to loss of water from the body that causes decreased extracellular fluid volume. There was also a decrease in $Cl^-$ concentration, which was interpreted to be secondary to a shift of $Na^+$. Metabolic alkalosis is closely linked to $Cl^-$ depletion, which leads to an increased reabsorption of $HCO_3^-$ in the distal renal tubule (Khanna and Kurtzman, 2001). Sodium ion and $Cl^-$ are the most abundant osmotically active solutes. However, the amount of $Na^+$ in the extracellular fluid is the most important. Based on the key position of $Na^+$ in volume homeostasis, more than 1 mechanism operates to control the excretion of this ion. Sodium deficits and extracellular volume contraction usually occur concurrently with $Cl^-$ depletion alkalosis, and because of this, there is some controversy regarding their individual contributions to the maintenance of $Cl^-$ (Galla and Luke, 1988). The acid-base status can also be assessed by calculating the anion gap using the formula of Guyton and Hall (2001), which is the difference in the levels of the major cations ($Na^+$ and $K^+$) and the major anions ($Cl^-$ and $HCO_3^-$) in blood. The anion gap is frequently decreased to a modest degree in metabolic alkalosis and respiratory acidosis, whereas it is elevated in metabolic acidosis and respiratory alkalosis (Carlson, 1989).

Birds fed the H DEB diet exhibited slightly lower $pCO_2$ than birds fed the M DEB diet in both the ACTH and control groups on d 42. In addition, the H DEB diet slightly elevated $pO_2$ on d 49 compared with control birds fed the M DEB diet. The levels of $pO_2$ were lower in the ACTH treatment groups because of an increased demand for oxygen to fulfill the required higher metabolic oxygen demand under stressful conditions (Lugar et al., 2003; Aftab and Khan, 2005; Olkowski et al., 2005). We conclude that DEB diets do not play a major role in alleviating stress induced by continuous ACTH infusion in broiler chickens. However, the current data are important to the poultry industry in understanding the physiological role of DEB under stressful conditions when chickens must not only maintain their acid-base balance, but must also compensate for lost electrolytes under stressful conditions to enable them to meet nutrient requirements for their metabolic needs. Overall, our data suggest that a DEB of 241 mEq/kg will partially alleviate ACTH-induced adaptive stress in broiler chickens.

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